Cerebral Ischemia

Subjects: Clinical Neurology
Contributor: David Díaz Díaz

Cerebral ischemia represents the third cause of death and the first cause of disability in adults. This process results from decreasing cerebral blood flow levels as a result of the occlusion of a major cerebral artery. This restriction in blood supply generates low levels of oxygen and glucose, which leads to a decrease in the energy metabolism of the cell, producing inflammation, and finally, neurological deterioration. Currently, blood restoration of flow is the only effective approach as a therapy in terms of ischemic stroke. However, a significant number of patients still have a poor prognosis, probably owing to the increase in the generation of reactive oxygen species (ROS) during the reperfusion of damaged tissue. Oxidative stress and inflammation can be avoided by modulating mitochondrial function and have been identified as potential targets for the treatment of cerebral ischemia. In recent years, the beneficial actions of flavonoids and polyphenols against cerebrovascular diseases have been extensively investigated. The use of resveratrol (RSV) has been shown to markedly decrease brain damage caused by ischemia in numerous studies. According to in vitro and in vivo experiments, there is growing evidence that RSV is involved in several pathways, including cAMP/AMPK/SIRT1 regulation, JAK/ERK/STAT signaling pathway modulation, TLR4 signal transduction regulation, gut/brain axis modulation, GLUT3 up-regulation inhibition, neuronal autophagy activation, and de novo SUR1 expression inhibition.

Keywords: blood–brain barrier ; brain ; ischemia ; neuroprotection ; oxidative stress ; polyphenols ; reperfusion ; resveratrol ; therapy

1. Introduction

Resveratrol (RSV) is a natural stilbene class of polyphenol (*trans*-3,5,4′-trihydroxystilbene) present in a large variety of vegetal species, such as grapes, mulberries, peanuts, and pomegranates [1]. In recent years, the scientific community has been very interested in this compound due to its broad range of potential biological activities. In particular, important therapeutic effects have been related to this polyphenol administration as antioxidant [2], anti-inflammatory [3], cardioprotective [4], and anti-carcinogenic [5], among others [6][7].

RSV has been studied for the treatment of multiple diseases such as obesity, diabetes, cardiovascular problems [8]. Additionally, evaluation of its effects as a therapeutic agent on neuroprotection and nerve regeneration has gained special relevance in recent years [9]. For instance, RSV could mediate nerve regeneration and motor repair of sciatic nerve crush injury in a rat model [10] as well as improve spinal cord injury [11].

Stroke is a cerebrovascular disease caused by the interruption of blood flow to the brain due to the blockage or rupture of a vessel. Approximately 80% of all strokes are ischemic, and there are limited therapies approved for the treatment of acute ischemic stroke. Several natural compounds have been used for the prevention of strokes [12] and others that produce a protective action on the cardiovascular system, including RSV [13][14]. For example, atherosclerosis is considered a risk factor for ischemic stroke. In this sense, RSV could inhibit the platelet activation and aggregation induced by collagen, adenosine diphosphate, and thrombin. The proposed mechanism involved the inhibition either of tissue factor gene expression or the synthesis of prothrombotic mediators [15]. Cerebral edema and intracranial hypertension are common complications of cerebral infarction and the major causes of mortality. A range of therapeutic agents that successfully target cerebral edema have been developed in animal studies, some of which have been assessed in clinical trials; between them, RSV has been proved as a therapeutic agent [16][17]. Although RSV is a highly hydrophobic molecule, the penetration to a membrane such as the blood–brain barrier (BBB) is extremely difficult. However, an alternative administration is via the nasal in the olfactory region, resulting in a more comfortable route for the patient [18]. RSV has several mechanisms of action linked to their effects against stroke, as the molecule interacts with a wide range of enzymes and receptors, improving the resistance to stress and reducing apoptosis [19].

Brain damage and neuronal death after an acute central nervous system (CNS) injury such as stroke are synergistically mediated by many pathophysiologic mechanisms that include oxidative stress, inflammation, and ionic imbalance, besides apoptosis. As was mentioned, the treatment with RSV has been shown to prevent or slow down many of these

pathological changes [20]. Polyphenols also exert neuroprotective actions when administered after stroke, indicating that these molecules may be helpful in the recovery of stroke patients [21] and neonatal hypoxic–ischemic brain injuries [22][23]. There are several recent reviews dealing with the neuroprotective mechanisms produced by natural polyphenolic compounds [18][24][25]. Antioxidants such as RSV generally work as ROS scavengers and metal chelators due to the presence of hydroxyl groups [26]. It has been proposed that beneficial actions of the activated Sonic hedgehog (Shh) signaling pathway, such as improved neurological function, decreased infarct volume, enhanced vitality, and reduced apoptosis of neurons after stroke, can be produced by RSV [27]. Wan et al. reported that RSV also provides neuroprotection by inhibiting phosphodiesterase (PDEs) and regulating the cyclic adenosine monophosphate (cAMP)/adenosine monophosphate (AMPK)/sirtuin 1 (SIRT1) pathway, which reduces ATP energy consumption during ischemia [28].

The effects of RSV in nerve regeneration after an ischemic brain injury episode are shown in **Figure 1**.

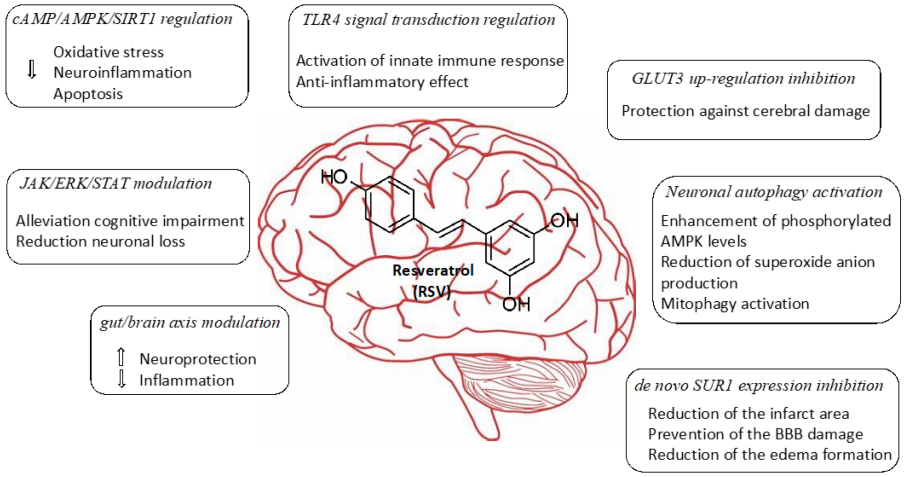

Figure 1. Potential mechanisms of action during an ischemic brain injury episode after treatment with RSV covered in this review.

2. Neuroprotective Mechanism Associated with Treatments Involving Resveratrol

2.1. Regulation of cAMP/AMPK/SIRT1 Pathway

Recently, dysfunction of energy metabolism has been considered as a possible important pathophysiological basis for cerebrovascular accidents. In this way, Wan et al. evaluated the neuroprotector action of RSV in cerebral ischemic injury [28]. Besides, the authors proposed a mechanism of action based on the regulation of energy metabolism, identifying potential targets of RSV. Results showed that treatment with RSV induced activation of AMPK and SIRT1 by inhibiting cyclic nucleotide PDEs.

For the development of the studies, in vivo cerebral ischemia injury was induced in 7–8-week-old male Sprague Dawley rats by middle cerebral artery occlusion (MCAO). After 2 h, the cerebral obstruction was removed, and rats were reperfused. RSV dissolved in 5% dimethyl sulfoxide was repeatedly injected via intraperitoneal at 20 mg/kg once a day for 5 days before the occlusion procedure and for the final time before surgery. Results suggested a neuroprotective effect of RSV in MCAO rats since the degree of neurological deficits (based on a 5-point scale) significantly decreased from 2.75 for the control group to 1.67 after RSV treatment (**Figure 2**).

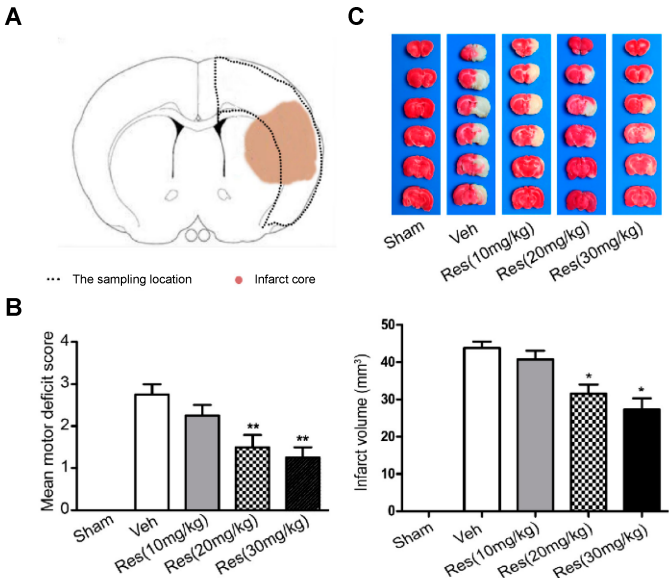

Figure 2. RSV provides neuroprotection 24 h after MCAO. (**A**) Schematic illustration of the sampling locations on the ipsilateral side of the cerebral cortex; (**B**) Neurological deficit scores following administration of 20 mg/kg and 30 mg/kg in the resveratrol treatment group were significantly decreased compared with the vehicle group; (**C**) 2,3,5-Triphenyltetrazolium chloride (TTC) staining of coronal sections and quantitative analysis of infarct regions 24 h after MCAO. Red and white represent normal and infarct tissue, respectively. Data are expressed as mean ± S.E.M. (* $p < 0.05$ and ** $p < 0.01$; $n = 4$ in each group). Adapted with permission from Elsevier (Wan, 2016).

2.2. Modulating JAK/ERK/STAT Signaling Pathway

To investigate the protective effect of RSV on hippocampus neurons from cerebral ischemia, Chang et al. administered RSV after an induced stroke. The authors proposed that RSV inhibited not only phosphorylation of Janus kinase (JAK)/extracellular signal-regulated kinases (ERK)/signal traducers and activators of transcription (STAT), exerting a neuroprotective effect, but also promoted downregulation of inflammatory cytokines, reduced neuronal loss, and alleviated cognitive impairment [29].

The authors carried out in vivo studies inducing a cerebral ischemia–reperfusion injury in Sprague Dawley rats. The occlusion was maintained for 2 h, and treatment started for 7 consecutive days using a concentration of 20 mg/kg of RSV dissolved in 50% propylene glycol. The locomotive activity of rats was evaluated using open-field and closed-field tests after 7 days. One week later, the authors studied both memory and learning abilities using the Morris water maze (MWM) test. Then, 3 weeks later after injury, rats were sacrificed to conduct histology, Western blot assay, lipid peroxidation, and antioxidant enzyme assays. This study demonstrated that RSV was able to protect the neuronal loss in the hippocampus from ischemia/reperfusion injury via modulating the JAK/ERK/STAT signaling pathway. This was confirmed using hematoxylin-eosin (HE) staining by counting the number of necrotic neurons in the hippocampus. In addition, the authors showed that RSV prevented memory deficits in rats and effectively reduced lipid peroxidation involved in programmed cell death cascades and inflammation. Therefore, these preliminary results have served to underscore the potential of RSV in treating ischemia–reperfusion injury in the brain, but further experiments should be properly designed to launch preclinical studies to confirm this capability.

2.3. Regulation of TLR4 Signal Transduction

Neonatal hypoxic–ischemic encephalopathy (HIE), or hypoxic–ischemic brain injury (HIBI), is caused by disorders in cerebral blood flow and oxygen supply, being the most common cause of perinatal brain injury, which can lead to neonatal death or cause irreversible or prolonged physical and mental disabilities that lack appropriate treatment. Le et al. evaluated the activity of high mobility group box-1 (HMGB1) through the activation of SIRT1 by RSV in the inhibition of HI insult-induced neuroinflammation [30]. Using in vivo and in vitro assays, the authors confirmed that RSV inhibited the acetylation level and release of HMGB1, which was actively involved in the activation of the inflammatory cytokines signaling pathways (Toll-Like Receptor 4 (TLR4)/MyD88/Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)), by increasing the expression and activity of SIRT1.

2.4. Mechanism Targeting Gut/Brain Axis

Inflammation and immune system activation are two important processes that can affect the brain after acute stroke. Importantly, emerging evidence has proved the role of the microbiota–gut brain axis in the ischemic brain. In this sense, Dou et al. demonstrated for the first time that RSV might inhibit systemic post-stroke inflammations, inducing neuroprotection by modulating intestinal-immune-cell-mediated inflammation [31]. The authors proposed one of the possible multifactorial mechanisms through which RSV could act is by modulating T helper 17 (Th17)/regulatory T (Treg) and Th1/Th2 cells as well as the expression of their related cytokines in the small intestine.

2.5. Inhibition of GLUT3 Up-Regulation

After cerebral ischemia, the brain metabolic energy is reduced. In consequence, an up-regulation of glucose transporter (GLUT3) is an adaptive response for the rapid nutrient supply. Nonetheless, the fluctuation in glucose levels alters mitochondrial functionality, promotes apoptosis, and stimulates pro-inflammatory factors. On this basis, Gutiérrez Aguilar et al. studied the effect of RSV on GLUT3 expression levels after induced ischemia [32]. Results showed that the polyphenol protects neurons from middle cerebral artery occlusion damage and prevents GLUT3 up-regulation in the injured brain that could depend on AMPK activation.

2.6. Activation of Neuronal Autophagy

Based on the fact that AMPK preserves the cellular energy levels and may contribute to neuroprotection by activating autophagy after cellular stress such as cerebral ischemia or excitotoxicity, Pineda-Ramírez et al. investigated the neuroprotector effect of resveratrol on this via [33]. They demonstrated that RSV enhanced AMPK activity and drove autophagy, suggesting an increase of survival neurons. Herein, cerebral ischemia was induced in Wistar rats through the MCAO for 2 h, and then reperfusion was induced. A single dose of RSV (1.8 mg/kg in water–ethanol 50% *v/v*) was administrated in the tail vein at the onset of reperfusion. Results showed that p-AMPK levels were significantly increased when RSV was administrated in comparison with the non-treated group. Besides, the infarct area detected was markedly reduced after treatment with RSV than for the control group (20.76 ± 1.67% and 28.08 ± 2.84%, respectively). The employment of an AMPK inhibitor, compound C ((6-[4-(2-piperidin-1-yl-ethoxy)-phenyl)]-3-pyridin-4-ylpyrrazolo [1,5-a]–pyrimidine), showed interference with the protective effect of RSV. For instance, the infarct area was reduced to only 25.7 ± 2.25%; the rat survival after 24 h of reperfusion was 57% and 90.47% for rats treated with RSV. This suggested that the neuroprotector effect of the polyphenol was partially related to AMPK activation.

2.7. Via De Novo SUR1 Expression Inhibition

Another potential mechanism in which RSV has shown effectiveness is in reducing the infarct area and cerebral edema, resulting in an improvement of the neurological performers, reduction of the blood–brain barrier (BBB) damage, and increase of the survival acting in the process of inhibiting Abcc8 gene transcription that codes for Sulfonylurea receptor 1 (SUR1).

2.8. Administration of Resveratrol-Loaded Nanoparticles (NPs)

In addition to using RSV itself as a therapeutic molecule to treat ischemic brain injuries, other strategies have been assessed. Lu et al. prepared NPs made up of poly(N-vinylpyrrolidone)-*b*-poly(ε-caprolactone) (PVP-PCL) as drug carriers for RSV [34]. The resulting particles exhibited spherical shape and diameters of 100 nm, according to Scanning Electron Microscopy (SEM) and Dynamic Light Scattering (DLS), respectively. In vitro controlled release experiments were performed. These assays showed an initial burst release of 37% and sustained release of RSV close to 65% after 6 days.

3. Conclusions

Herein we collected various recent studies describing the evaluation of the RSV effectiveness in the treatment of ischemic brain injury in different rodent animal models. RSV itself shares common properties with other polyphenol derivatives such as neuroprotection, anti-apoptotic, anti-inflammatory, and antioxidant characteristics. This has allowed RSV to be considered a promising small molecule drug for the treatment of age-related disorders. In this regard, data experiments collected from recent studies have shown different mechanisms of action in which RSV plays important roles in the management of the disease, such as cAMP/AMPK/SIRT1 regulation, JAK/ERK/STAT signaling pathway modulation, TLR4 signal transduction regulation gut/brain axis modulation, GLUT3 up-regulation inhibition, neuronal autophagy activation, and de novo SUR1 expression inhibition. The in vivo experiments that have been selected for this review may open novel perspectives and challenges for ischemic brain injuries with the potential to be translated from bench to bedside. While

RSV has exhibited remarkable activities when it has been systemically administered, its poor bioavailability makes this drug unsuitable for clinical use. Interestingly, novel encapsulation strategies and the use of nanotechnology have emerged to overcome these drawbacks and might facilitate the transport of RSV at a specific site of action. Despite the fact that there are still no effective drugs approved for the treatment of cerebral ischemia, additional and detailed preclinical experiments should be performed involving a range of animal models with the aim to afford the most efficient therapy strategies, including new encapsulation techniques and the use of RSV-load NPs.

References

1. Summerlin, N.; Soo, E.; Thakur, S.; Qu, Z.; Jambhrunkar, S.; Popat, A. Resveratrol nanoformulations: Challenges and opportunities. Int. J. Pharm. 2015, 479, 282–290.
2. Diaz-Gerevini, G.T.; Repossi, G.; Dain, A.; Tarres, M.C.; Das, U.N.; Eynard, A.R. Beneficial action of resveratrol: How and why? Nutrition 2016, 32, 174–178.
3. Singh, A.P.; Singh, R.; Verma, S.S.; Rai, V.; Kaschula, C.H.; Maiti, P.; Gupta, S.C. Health benefits of resveratrol: Evidence from clinical studies. Med. Res. Rev. 2019, 39, 1851–1891.
4. Raj, P.; Lieben Louis, X.; Thandapilly, S.J.; Movahed, A.; Zieroth, S.; Netticadan, T. Potential of resveratrol in the treatment of heart failure. Life Sci. 2014, 95, 63–71.
5. Elshaer, M.; Chen, Y.; Wang, X.J.; Tang, X. Resveratrol: An overview of its anti-cancer mechanisms. Life Sci. 2018, 207, 340–349.
6. Delmas, D.; Cornebise, C.; Courtaut, F.; Xiao, J.; Aires, V. New highlights of resveratrol: A review of properties against ocular diseases. Int. J. Mol. Sci. 2021, 22, 1295.
7. Kulkarni, S.S.; Cantó, C. The molecular targets of resveratrol. Biochim. Biophys. Acta 2015, 1852, 1114–1123.
8. Rauf, A.; Imran, M.; Suleria, H.A.R.; Ahmad, B.; Petersf, D.G.; Mubarak, M.S. A comprehensive review of the health perspectives of resveratrol. Food Funct. 2017, 8, 4284–4305.
9. Huang, J.; Huang, N.; Xu, S.; Luo, Y.; Li, Y.; Jin, H.; Yu, C.; Shi, J.; Jin, F. Signaling mechanisms underlying inhibition of neuroinflammation by resveratrol in neurodegenerative diseases. J. Nutr. Biochem. 2021, 88, 108552.
10. Ding, Z.; Cao, J.; Shen, Y.; Zou, Y.; Yang, X.; Zhou, W.; Guo, Q.; Huang, C. Resveratrol promotes nerve regeneration via activation of p300 acetyltransferase-mediated VEGF signaling in a rat model of sciatic nerve crush injury. Front. Neurosci. 2018, 12, 341.
11. Zhang, S.; Botchway, B.O.A.; Zhang, Y.; Liu, X. Resveratrol can inhibit Notch signaling pathway to improve spinal cord injury. Ann. Anat. 2019, 223, 100–107.
12. Tang, K.S.; Tan, J.S. The protective mechanisms of polydatin in cerebral ischemia. Eur. J. Pharmacol. 2019, 842, 133–138.
13. Tressera-Rimbau, A.; Arranz, S.; Eder, M.; Vallverdú-Queralt, A. Dietary polyphenols in the prevention of stroke. Oxid. Med. Cell. Longev. 2017, 2017, 7467962.
14. Zou, L.; Gong, Y.; Liu, S.; Liang, S. Natural compounds acting at P2 receptors alleviate peripheral neuropathy. Brain Res. Bull. 2019, 151, 125–131.
15. Cheng, Y.C.; Sheen, J.M.; Hu, W.L.; Hung, J.C. Polyphenols and Oxidative Stress in Atherosclerosis-Related Ischemic Heart Disease and Stroke. Oxid. Med. Cell. Longev. 2017, 2017, 8526438.
16. Yao, Y.; Zhang, Y.; Liao, X.; Yang, R.; Lei, Y.; Luo, J. Potential therapies for cerebral edema after ischemic stroke: A mini review. Front. Aging Neurosci. 2021, 12, 618819.
17. Shao, A.; Lin, D.; Wang, L.; Tu, S.; Lenahan, C.; Zhang, J. Oxidative stress at the crossroads of aging, stroke and depression. Aging Dis. 2020, 11, 1537–1566.
18. Long, Y.; Yang, Q.; Xiang, Y.; Zhang, Y.; Wan, J.; Liu, S.; Li, N.; Peng, W. Nose to brain drug delivery—A promising strategy for active components from herbal medicine for treating cerebral ischemia reperfusion. Pharmacol. Res. 2020, 159, 104795.
19. Parrella, E.; Gussago, C.; Porrini, V.; Benarese, M.; Pizzi, M. From preclinical stroke models to humans: Polyphenols in the prevention and treatment of stroke. Nutrients 2021, 13, 85.
20. López, M.; Dempsey, R.J.; Vemuganti, R. Resveratrol neuroprotection in stroke and traumatic CNS injury. Neurochem. Int. 2015, 89, 75–82.

21. Chen, S.; Chen, H.; Du, Q.; Shen, J. Targeting myeloperoxidase (MPO) mediated oxidative stress and inflammation for reducing brain ischemia injury: Potential application of natural compounds. Front Physiol. 2020, 11, 433.
22. Mohsenpour, H.; Pesce, M.; Patruno, A.; Bahrami, A.; Pour, P.M.; Farzaei, M.H. A review of plant extracts and plant-derived natural compounds in the prevention/treatment of neonatal hypoxic-ischemic brain injury. Int. J. Mol. Sci 2021, 22, 833.
23. Reyes-Corral, M.; Sola-Idígora, N.; de la Puerta, R.; Montaner, J.; Ybot-González, P. Nutraceuticals in the prevention of neonatal hypoxia–ischemia: A comprehensive review of their neuroprotective properties, mechanisms of action and future directions. Int. J. Mol. Sci. 2021, 22, 2524.
24. Subedi, L.; Gaire, B.P. Phytochemicals as regulators of microglia/macrophages activation in cerebral ischemia. Pharmacol. Res. 2021, 165, 105419.
25. Rahimifard, M.; Maqbool, F.; Moeini-Nodeh, S.; Niaz, K.; Abdollahi, M.; Braidy, N.; Nabavi, S.M.; Nabavi, S.F. Targeting the TLR4 signaling pathway by polyphenols: A novel therapeutic strategy for neuroinflammation. Ageing Res. Rev. 2017, 36, 11–19.
26. Tresserra-Rimbau, A.; Lamuela-Raventos, R.; Moreno, J.J. Polyphenols, food and pharma. Current knowledge and directions for future research. Biochem. Pharmacol. 2018, 156, 186–195.
27. Yu, P.; Wang, L.; Tang, F.; Zeng, L.; Zhou, L.; Song, X.; Jia, W.; Chen, J.; Yang, Q. Resveratrol pretreatment decreases ischemic injury and improves neurological function via Sonic Hedgehog signaling after stroke in rats. Mol. Neurobiol. 2017, 54, 212–226.
28. Wan, D.; Zhou, Y.; Wang, K.; Hou, Y.; Hou, R.; Ye, X. Resveratrol provides neuroprotection by inhibiting phosphodiesterases and regulating the cAMP/AMPK/SIRT1 pathway after stroke in rats. Brain Res. Bull. 2016, 121, 255–262.
29. Chang, C.; Zhao, Y.; Song, G.; She, K. Resveratrol protects hippocampal neurons against cerebral ischemia-reperfusion injury via modulating JAK/ERK/STAT signaling pathways in rats. J. Neuroimmunol. 2018, 315, 9–14.
30. Le, K.; Daliv, E.C.; Wu, S.; Qian, F.; Ali, A.I.; Yu, D.; Guo, Y. SIRT1-regulated HMGB1 release is partially involved in TLR4 signal transduction: A possible anti-neuroinflammatory mechanism of resveratrol in neonatal hypoxic-ischemic brain injury. Int. Immunopharmacol. 2019, 75, 105779.
31. Dou, Z.; Rong, X.; Zhao, E.; Zhang, L.; Lv, Y. Neuroprotection of resveratrol against focal cerebral ischemia/reperfusion injury in mice through a mechanism targeting gust-brain axis. Cell. Mol. Neurobiol. 2019, 39, 883–898.
32. Gutiérrez Aguilar, G.F.; Alquisiras-Burgos, I.; Franco-Pérez, I.; Pineda-Ramírez, N.; Ortiz-Plata, A.; Torres, I.; Pedraza-Chaverri, J.; Aguilera, P. Resveratrol prevents GLUT3 up-regulation induced by middle cerebral artery occlusion. Brain Sci. 2020, 10, 651.
33. Pineda-Ramírez, N.; Alquisiras-Burgos, I.; Ortiz-Plata, A.; Ruiz-Tachiquín, M.E.; Espinoza-Rojo, M.; Aguilera, P. Resveratrol activates neuronal autophagy through AMPK in the ischemic brain. Mol. Neurobiol. 2020, 57, 1055–1069.
34. Lu, X.; Dong, J.; Zheng, D.; Li, X.; Ding, D.; Xu, H. Reperfusion combined with intraarterial administration of resveratrol-loaded nanoparticles improved cerebral ischemia-reperfusion injury in rats. Nanomed. Nanotech. Biol. Med. 2020, 28, 102208.